33. A method of cloning sequences (target sequences) each containing a sequence encoding at least part of an immunoglobulin variable domain, which method comprises

providing a sample repertoire of nucleic acid containing target sequences, and

using forward and back primers in the copying and cloning of the target sequences for expression of a repertoire of proteins each comprising at least part of an immunoglobulin variable domain, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of each of the target sequences, the back primer being specific for a sequence at or adjacent the 3' end of the antisense strand of each of the target sequences.

34. A method according to claim 33 which method comprises:

(a) providing a sample repertoire of double-stranded nucleic acid containing target sequences;

(b) causing the two strands of the double-stranded nucleic acid to be separated;

(c) annealing to the sample a forward and a back oligonucleotide primer, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of each of the target sequences, the back primer being specific for a sequence at or adjacent the 3' end of the antisense strand of each of

the target sequences, under conditions which allow the primers to hybridize specifically to the nucleic acid;

(d) treating the annealed sample with a DNA polymerase enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place, thereby producing double-stranded nucleic acid;

(e) repeating steps (b) to (d), thereby producing some double-stranded DNA (product DNA) containing only the target sequences;

(f) cloning product DNA into expression vectors for expression of a repertoire of proteins each comprising at least part of an immunoglobulin variable domain.

35. A method according to claim 34 wherein steps (b) to (d) are repeated a plurality of times before step (f).

36. A method according to claim 33, which comprises:

(a) providing a repertoire of mRNA;

(b) annealing to the mRNA an oligonucleotide primer specific for a sequence at or adjacent the 3' end of each of the target sequences on the sense strands, under conditions which allow the primer to hybridize specifically to the nucleic acid;

(c) treating the primer-annealed mRNA with a polymerase enzyme in the presence of deoxynucleoside

cont C1

triphosphates under conditions which cause primer extension to take place, thereby producing antisense cDNA;

(d) annealing to the cDNA an oligonucleotide primer specific for a sequence at or adjacent the 3' end of each of the target sequences on the antisense strands, under conditions which allow the primer to hybridize specifically to the nucleic acid;

sub C1 cont

(e) treating the primer-annealed cDNA with a polymerase enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place, thereby producing double-stranded DNA (product DNA);

(f) cloning product DNA into expression vectors for expression of a repertoire of proteins each comprising at least part of an immunoglobulin variable domain.

37. A method according to claim 36 wherein, after step (e) the following steps are performed before step (f);

(i) causing the two strands of the product DNA to be separated;

sub C2

(ii) annealing to the separated strands a forward and a back oligonucleotide primer, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of each of the target sequences, the back primer being specific for a

sequence at or adjacent the 3' end of the antisense strand of each of the target sequences, under conditions which allow the primers to hybridize specifically to the nucleic acid;

(iii) treating the annealed sample with a DNA polymerase enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place, thereby producing double-stranded nucleic acid.

38. A method according to claim 33 wherein the back primer is specific for a sequence at or adjacent the 3' end of the antisense strand of the sequences which are contained in the target sequences and which each encode at least part of an immunoglobulin variable domain.

39. A method according to claim 33 wherein ~~the sample~~ said repertoire of double-stranded nucleic acid is derived from lymphocytes.

40. A method according to claim 39 wherein the lymphocytes are derived from an animal or human mounting an immune response to an antigen.

41. A method according to claim 39 wherein the lymphocytes are derived from a patient with an autoimmune disease.

42. A method according to claim 33 wherein ~~the sample~~ said repertoire of nucleic acid sequences is derived from rearranged immunoglobulin variable region genes.

43. A method according to claim 33 wherein ~~the sample~~ repertoire of nucleic acid sequences is genomic DNA.

44. A method according to claim 33 wherein ~~the sample~~ repertoire of nucleic acid sequences is derived from unrearranged immunoglobulin variable region genes.

45. A method according to claim ~~33~~ 34 wherein ~~the target~~ said sequence contains a sequence encoding a variable domain from an immunoglobulin heavy chain.

46. A method according to claim 45 wherein ~~the~~ said product DNA is inserted into an expression vector for expression of single domain ligands selectable by their binding affinity for antigen.

47. A method according to claim ~~33~~ 34 wherein said product DNA is inserted into an expression vector for expression of antibodies or antibody fragments selectable by their binding affinity for antigen.

48. A method according to claim ~~33~~ 34 wherein ~~the~~ said product DNA is inserted into an expression vector for expression alone.

49. The method of claim ~~33~~ 34 wherein ~~the~~ said product DNA is inserted into an expression vector for expression in combination with a complementary variable domain.

50. A method according to claim ~~33~~ 34 wherein ~~the~~ said product DNA is inserted into an expression vector already containing sequences encoding one or more constant domains for expression.

51. A method according to claim ~~33~~ 34 wherein ~~the~~ said product DNA is inserted into an expression vector for expression as fusion proteins.

52. A method according to claim ~~33~~ 34 wherein ~~the~~ said product DNA is inserted into an expression vector for expression with peptide tags.

53. A method according to claim ~~33~~ 34 wherein said product DNA containing sequences encoding at least immunoglobulin heavy chain variable domains is inserted into expression vectors along with product DNA containing sequences encoding at least immunoglobulin light chain variable domains for expression of a combinatorial repertoire of complementary variable domains.

54. A method according to claim 53 wherein ~~the~~ said product DNA is inserted into an expression vector already containing sequences encoding one or more constant domains for expression.

55. A method according to claim 53 wherein said product DNA is inserted into expression vectors for expression as fusion proteins.

56. A method according to claim 53 wherein ~~the~~ said product DNA is inserted into an expression vector for expression with peptide tags.

57. A method according to claim 33 wherein the forward and back primers are provided as single oligonucleotides.

58. A method according to claim 33 wherein ~~the~~ said forward ~~primers are~~ primer is supplied as a mixture of oligonucleotides.

59. A method according to claim 33 wherein ~~the~~ said back ~~primers are~~ primer is supplied as a mixture of oligonucleotides.

60. A method according to claim 33 wherein each primer includes a sequence encoding a restriction enzyme recognition site.

61. A method according to claim 60 wherein the restriction enzyme recognition site is located in the sequence which is annealed to the nucleic acid.

Cont E

62. A method according to claim 33 wherein ~~the~~ said back primer is a general primer useful for cloning a desired antibody specificity from a specific species.

C 1

E

63. A method according to claim 33 wherein ~~the~~ said back primer is a mixture of primers having a variety of sequences designed to be complementary to the various families of VH, Vk or Vλ sequences.